## Induction of uncoiled chromosomes by vibration[1]

J. G. Delinassios

*Hellenic Anticancer Institute, Papanicolaou's Research Center, Department of Biochemistry, 171, Alexandras Avenue, Athens 603 (Greece), 30 May 1978*

*Summary.* Chromatin condensation during metaphase can be removed by simple vibration of metaphase cells prior to fixation. Uncoiled chromosome arms consist of long threads with dense regions at irregular distances each from the other.

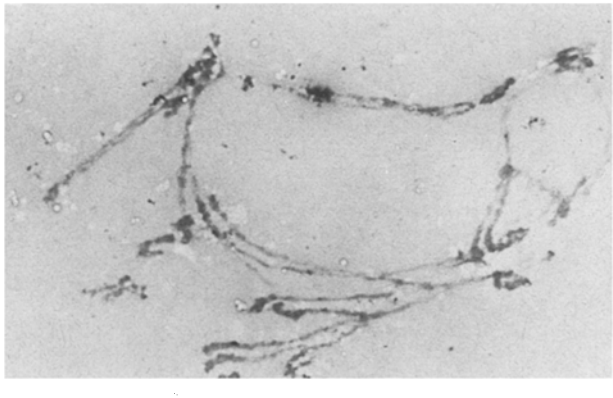
The various procedures for producing banding patterns in metaphase chromosomes suggest that along the chromosome arms exist regions with different properties[2]. Additionally, electron microscopy of whole chromosomes has shown the high complexity of the structure and the presence of perichromosomal fibrils as a main feature, although it failed to indicate any distinct structural difference between banded and nonbanded regions[3–6]. Considering the coiled structure of mammalian chromosome arms[7–9], it was thought that a procedure involving uncoiling, fixation and staining for light or electron microscopy might give a better view of the chromosome structure. In this report, vibration of metaphase cells prior to fixation is proposed as a method to induce uncoiled and super-uncoiled chromosomes.

*Methods.* A clone of Chinese hamster fibroblasts, CH-23[10], grown in Hám's F12 medium was used. Cells were treated with colchicine 0.06 µg/ml for 3–5 h and metaphase cells were selectively detached from the glass surface by shaking-trypsinization for 1 min, with cold 0.025% trypsin solution, pH 7.8[11]. Harvested cells were washed twice in physiological saline and suspended in a hypotonic solution of 1% sodium citrate, pH 7.5, at a density between $2\times10^5$ and $1\times10^6$ cells/ml, and left at 37 °C for 10 min. The cell suspension was then vibrated for 5 sec to 1 min on a Vortex-Genie Vibrator, model K-550-GE (Springfield Sci. Industries Inc., Mass., USA) at speed control 5; subsequently, it was centrifuged at 70 × g for 4 min. The cell pellet was dispersed in freshly prepared fixative (ethanol: glacial acetic acid, 3:1), and drops of it were immediately applied on alcohol-washed slides. Uncoiled fixed chromosomes showed high affinity for the glass surface; therefore, the cell suspension was immediately applied on the slides by means of a broad-end pasteur pipette. Preparations were stained with acetic orcein (Gurr, London, England) for 10–20 min, washed and mounted.

*Results.* Vibrated metaphase chromosomes undergo structural changes: Figures 1 and 2 show the profound uncoiling of Chinese hamster chromosomes after vibration for 30 and 60 sec prior to fixation, respectively. Prolonged vibration produces complete despiralization of the chromosomes, which become extremely glutinous and tend to form easily aggregates. After the 30-sec vibration time, each chromosome arm is shown to consist of a long super-coiled thread, forming a helix with highly condensed turnings (figure 1). Vibration for 60 sec and subsequent cell lysis and disruption of the metaphase spindle gave rise to further uncoiling of the chromosomes and appearance of long thin threads exhibiting dense dark spot regions at irregular distances (figure 2). Uncoiled chromosomes as those observed by Ohnuki[8] were found mainly isolated on the slides, rarely observed within a metaphase plate. No uncoiling effect on the chromosomes on nonvibrated cells was observed (figure 3).

Vibration causes: 1. Disruption of the cellular membrane in most of the metaphase cells after hypotonic treatment. 2. Release of the chromosomes in suspension and dispersion of the spindle system. 3. Super-uncoiling and complete despiralization of the chromosomes. It seems that lysis of the external cellular membrane is necessary for complete chromosome uncoiling.

*Discussion.* At present, it is not known how vibration forces act on the chromosomes and which components are affected. Electron microscopy of whole mount chromosomes revealed that condensed chromosomes appear as highly complicated packed fibres[5,12], and under certain preparation conditions they exhibit a clear distinction into band and interband regions, even without staining[3,6,13]; these results should prevent any simplification of a proposed model. It should however be accepted that the process of unwinding-uncoiling starts after the breakage of an intrachromosomal system of connective forces which preserve chromosome contraction during metaphase. The potential of this system is increased by colchicine or vincristine and decreased by daunomycin or cycloheximide[9], hypotonic treatment[8], heat[14] and detergent treatment[7]. These intrachromosomal forces are attributed to the formation of perichromosomal fibrils[9] and cannot be abolished by simple isolation of the chromosomes from their cellular environment; thus, isolated chromosomes show normal contraction[12]. It is proposed that the factor which is responsible for



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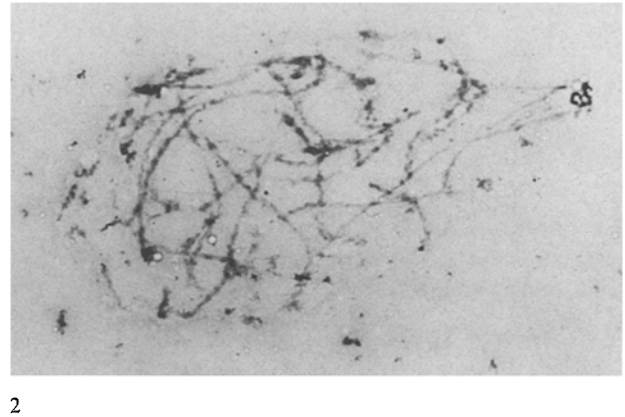

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Figures 1 and 2. Effect of vibration in hypotonic solution prior to fixation on Chinese hamster metaphase chromosomes. Metaphase cells were vibrated for 30 sec (1) and 60 sec (2), fixed and stained with acetic orcein.

chromosome condensation in metaphase can be removed by vibration of metaphase cells in hypotonic solution prior to fixation.

From the present results, it is inferred that uncoiled chromosome arms show irregularity in their capacity to absorb acetic orcein along their length. The observation of dense regions at irregular distances from each other along the arms indicates that contraction of the spiralized chromatin in metaphase chromosomes conceals details along the chromatid thread. On the other hand, these dense regions may affect the banding patterns of contracted fixed chromosomes; on this line, differential condensation of the chromatid spirals has already been proposed as an explanation of banding patterns[7,13]. In favor of this aspect, adequate evidence has accumulated showing that agents causing banding (e.g. SDS[15]; urea[16]; heat[17]; daunomycin[18]) may also cause despiralization under different conditions (SDS, urea[7]; heat[14]; daunomycin[9]).

In comparison with other methods for producing uncoiled chromosomes[8,9,14], vibration provides speed and elimination of chemical treatments. A combination of the uncoiling effect of vibration with biochemical techniques may be useful in the elucidation of the general chromosome architecture.

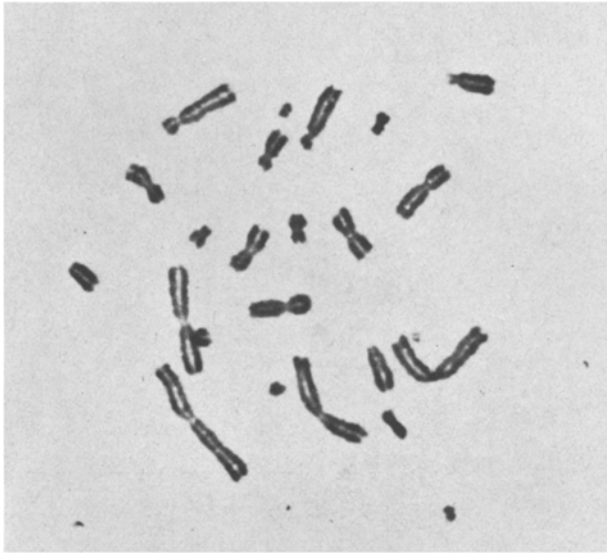

Fig. 3. A nonvibrated Chinese hamster metaphase cell.

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## Changes in glutamine levels during starvation and aestivation in the Indian apple snail *Pila globosa* (Swainson)

C. Sree Ramulu Chetty[1], R. Chandramohan Naidu, P. Aruna and K.S. Swami

*Department of Zoology, S.V. University, Tirupati 517502 (India), 2 June 1978*

*Summary.* The glutamine levels in digestive gland, foot and body fluids of normal, starved and aestivated *Pila globosa* were estimated. Glutamine content was decreased during starvation and aestivation. The percent decrease was more during aestivation than starvation. The decrease may be due to low glutamine-synthetase activity or increased utilization for uric acid synthesis. This may have an adaptive value during aestivation and starvation.

The Indian apple snail, *P. globosa*, represents an interesting case where the animal is ammonotelic in its active life and uricogenic during aestivation[2]. The urea and ammonia levels decrease in digestive gland, foot and body fluids, while uric acid levels increased several fold in the tissues and body fluid in aestivating *P. globosa*[3,4]. Similar changes were observed in chicken during fasting[5].

Though the synthesis of glutamine plays an important role in the detoxification of ammonia through the mediation of glutamate dehydrogenase and glutamine synthetase[6], this aspect of metabolism has received scant attention in molluscs[7]. Earlier reports have shown a gradual decrease in glutamine synthetase activity during the course of aestivation[8] and during starvation stress. Hence, it is necessary to estimate the levels of glutamine in digestive gland, foot and body fluids of active, starved and aestivated snails and to see whether it has any bearing in starvation and aestivation metabolism.

Collection, maintenance of snails and mode of aestivation were described elsewhere[3]. A batch of active snails were kept in water without food for 1 month. Starved animals and snails aestivated for 1 year were used in the present investigation. Digestive gland and foot were isolated in cold and a 10% homogenate of each tissue was prepared in ice-cold distilled water. The homogenates were centrifuged at 3000 rpm for 10 min to remove the cell debris and 0.1 ml of the centrifuged homogenates and body fluid were subjected to glutamine assay, employing the method described by Wilcox[9], and the liberated ammonia was estimated by Nesslerization.

The glutamine content showed a decrease in the tissue and body fluids of both starved and aestivated snails (table).